Fig. A1 is a depiction of in vitro proliferation of keratinocytes on plain LASERSKIN™ artificial skin showing holes for drainage of exudate (x100).

Fig. A2 is a depiction of a Type A modified Composite Biocompatible Skin Graft (CBSG) according to the invention wherein allogenic fibroblasts were seeded on both sides of the LASERSKIN™ artificial skin (x400).

Fig. A3 is a depiction of a Type B modified Composite Biocompatible Skin Graft (CBSG) according to the invention wherein fibroblast was seeded only on the side opposite the basal side of the LASERSKIN™ artificial skin (x400).

In addition, Applicants have filed herewith one sheet of corrected drawings of Figs. 2A-2C with a transmittal letter addressed to the Official Draftsperson. The revised Figs. 2A-2C add no new matter and correct line appearance and shading.

REMARKS

1. Status of the Claims:

Claims 1-6, 8-13, and 17-30 are presented for examination. Claims 7, 14, and 16 have been canceled. Claims 1, 4, 8, 11, and 15 have been amended. Claims 18-30 are newly added. The pending claims are set forth in the Appendix for the Examiner's convenience.

Claims 15-17 stand rejected as allegedly not enabled.

Claims 1-17 stand rejected as allegedly indefinite on various grounds.

Claims 1, 7, 8 and 14 stand rejected as allegedly anticipated by Della Valle *et al.* (1997).

Claims 2, 3, 9, and 10 stand rejected as allegedly obvious in view of Della Valle *et al.* (1997) and Hansbrough *et al.* (1989).

Claims 11-13 stand rejected as allegedly obvious in view of Della Valle *et al.* (1997) in view of Cooper *et al.* (1993), Hansbrough *et al.* (1989), and Meyers *et al.* (1977)

Applicants respectfully reply to these rejections below.

2. The Proposed Amendments to the Claims:

The proposed amendments to the claims would add no new matter.

Claims 1, 4, 11, and 15 have been amended to recite "human patient" and/or "human keratinocytes" and "human fibroblasts." Support for the human subject matter is found in the specification, for instance, in the paragraph bridging pp. 13 and 14.

Claims 1, 8, and 11 have been amended to recite "viable human keratinocytes." Support for the recital of "viable" keratinocytes is inherent in the process of growing the artificial skin and is also supported throughout the specification, and particularly, at line 24, p. 9.

Claims 1, 8, 11, and 15 have been amended to recite "viable human dermal fibroblasts." Support for the recital of "viable human dermal fibroblasts" is inherent in the process of growing the cells to subconfluence before seeding with the keratinocytes and is found throughout the specification.

Claims 1, 4, and 8 have been further amended for purposes of clarity to recite "for application to a human patient." This recital provided an antecedent basis to simplify the wording of the elements of the respective claims and/or dependent claims. These claims were further amended to recite "said patient" for "a target donor patient."

Claim 15 has been amended to recite "applying a basal side of a sheet of cultivated skin material over said collagen- glycoaminoglycan, said cultivated skin material comprising a layer of keratinocytes overlying a layer of viable human dermal fibroblasts upon an upper side of a biosynthetic substratum," This amendment to claim 15 is supported by the subject matter of canceled dependent claim 16.

New claims 18-20 are analogous to claims 15-17 except that they are more narrowly drawn to a biosynthetic substratum "of an esterified hyaluronic acid." Support for this subject matter is found for instance in the use of LASERSKIN™ artificial skin as exemplified throughout the application.

New claims 21-25 have been drawn to substratum of " a membrane comprising benzyl esterified hyaluronic acid." Support for this subject matter is found in the claims as originally filed.

New claims 26-30 depend respectively from new claims 21-25 and further recite "wherein the membrane has microholes." Support for this subject matter is found in the specification. LASERSKIN™ artificial skin has such holes as shown in photograph A1 and its legend as well as elsewhere in the specification. The enclosed Lam *et al.* reference

(incorporated by reference) describes the micropores of LASERSKIN™ artificial skin on p. 918, at the top of the second column of the main article.

As the foregoing amendments to the claims and newly added claims add no new subject matter, Applicants respectfully request that they be entered.

3. Status of the Specification:

The specification stands objected to for inappropriate usage of trademarks.

4. The Proposed Amendments to the Specification:

The proposed amendments to the specification add no new subject matter.

5. The Abstract:

The Abstract was amended to replace the recital of "Laserskin" with the recital of "artificial skin." The original specification (e.g., claim 1) disclosed other artificial skin materials than LASERSKIN™ artificial skin.

6. The Drawings:

The description of the figures A1 through A3 in Appendix A was amended to substitute "LASERSKIN™ artificial skin" for "Laserskin."

AMENDMENTS

I. The Description

The amendments to the specification as listed from A through L substituted the recital of "LASERSKIN™ artificial skin" for "Laserskin" wherever the latter recital appeared.

The amendments to the specification as listed from A through L substituted the recital of "INTEGRA™ artificial skin" or "INTEGRA™ neodermis" for "Integra" throughout.

In addition, Amendment A to the specification corrected the usage of the ALLODERM™ mark and added a generic description of the ALLODERM™ product as taught at the manufacturers web site (see enclosed print out).

In addition, in Amendment B, the references to "Integra™" were deleted.

In Amendment C, a generic description of LASERSKIN™ artificial skin was added. The description is not new matter. Support appears at the top of the second column of the

first page of the Lam *et al.* reference (Reference 10 on p. 3). This reference was incorporated by reference at p. 2, lines 26-28. One of ordinary skill in the art would well appreciate the construction of LASERSKIN™ artificial skin as evidenced in this art and the other art cited in the application.

Amendment D further inserts after the first sentence of the original Summary, the subject matter of the originally filed set of claims. The wording of the claims was edited to an appropriate paragraph style over the several paragraph inserts. The remainder of the original first paragraph follows the inserted paragraphs and has been amended to incorporate the above generic description of the LASERSKIN™ artificial skin product to correct the previous "Laserskin" usage. In addition, Amendment D provided the citation for the "Green method" recited therein. This reference was incorporated by reference on p. 2 as reference 1.

Amendment F deleted the recitals of "Laserskin" and "Integra" in the subject paragraph.

Amendment G substituted the recital "Biosynthetic biocompatible substratum" for the recital of "Laserskin" which accords with its description in the ensuing paragraph.

The proposed amendments to the specification add no new matter and Applicants respectfully request their entry.

II. Claims

A. Response to the Rejections Under 35 U.S.C. §112, first paragraph

1. Claims 15 –17

Claim 15 and its dependent claims 16 and 17 stand rejected as allegedly not being enabled with respect to the scope of the subject matter related to the "biosynthetic substratum." The Action states that the "take" rates are unpredictable when using different materials in composite skin grafts and using varying cell types and thus the claims are considered as allegedly not enabled in their scope. Applicants respectfully disagree.

In support thereof, the Action cited Cooper *et al.* as showing significant advantages of the composite graft over the epidermal sheet graft in the closure of full-thickness wounds. However, this evidence is simply not germane and cannot support the Action's use of it. Both the Cooper *et al.* composite graft and epidermal sheet graft were described in fact as being operable for their intended purpose. Moreover, Cooper *et al.* compared just one type of composite graft material to just one type of epithelial sheet. Cooper *et al.* describes nothing

about the variation in take rate for various biosynthetic substratum materials. They tested only one.

In addition, the test of enablement is not whether a claimed composite material is better than another composite material or whether they are uniformly operable or even optimized, but whether the claimed composites can be used for their intended purpose. In this respect, all that Cooper *et al.* reasonably teaches is that both a composite graft material and an epithelial sheet material, both differing from Applicants' claimed invention, are also operable.

In fact, the Della Valle *et al.* U.S. Patent cited by the Action as anticipating the invention discloses and claims a wide variety of biosynthetic biocompatible substratum for cultivating keratinocytes. For instance, Della Valle *et al.* disclose the following as suitable substratum:

> These membranes can consist of biocompatible and preferably also bioreabsorbable materials of natural origin such as collagen or coprecipitates of collagen and glycosaminoglycans, cellulose, gelled polysaccharides such as chitin, chitosan, pectins or pectic acids, agar, agarose, xanthan gum, gellan, alginic acid or alginates, polymannans or polyglucans, starches, or natural rubbers, either alone or in mixture with each other or with polymers of synthetic or semisynthetic origin, in the presence of suitable precipitating or gelling agents such as metal salts, polycations or polyanions.
>
> The membranes can also consist of biocompatible and preferably also bioreabsorbable materials of synthetic origin such as polylactic acid, polyglycolic acid or copolymers thereof or their derivatives, polydioxanones, polyphosphazenes, polysulphones or polyurethanes, or semisynthetic derivatives of natural polymers such as collagen crosslinked with crosslinking agents such as dialdehydes or their precursors, bicarboxylic acids or halides thereof, diamines, derivatives of cellulose, of alginic acid, of starch, of chitin chitosan, of gellan, of xanthan, of pectins or pectic acids, of polyglucans, of polymannans, of agar, of agarose, of natural rubbers or of glycosaminoglycans.

(see col. 3 first two paragraphs). Thus, one of ordinary skill in the art had a number of suitable biocompatible substratum available to them at the time the application was filed.

With respect to the alleged diversity of the cell types encompassed by these rejected claims, base claim 15 has been amended to recite "human" fibroblasts and "human" keratinocytes. The importance of interspecies variation to any selection of a suitable biocompatible substratum is therefore mooted.

Clearly, in view of these amendments, the claimsare fully enabled and, as such, Applicants respectfully request that the above rejections be reconsidered and withdrawn.

B. Response to the Rejections Under 35 U.S.C. §112, second paragraph

1. Claims 1-17

Claims 1-17 stand rejected as allegedly indefinite for the recital of "target donor patient." Applicants have amended the claims to avoid this recital. Applicants believe the proposed amendments moot this issue and respectfully request that the rejection be reconsidered and withdrawn.

2. Claims 1-6, 8-13, and 15-17

Claims 1-6, 8-13, and 15-17 stand rejected as allegedly indefinite with respect to the recitals of "layer" and "over" throughout the claims. The Action cited p. 11, paragraph 1 of the previous response as stating that "the keratinocytes and dermal fibroblasts according to the invention are in the same plane." However, the previous Amendment was discussing the Hansbrough *et al.* reference which awkwardly described the use of a laminated membrane to provide a "planar surface" for the cultured human keratinocytes (see, Hansbrough *et al.* p. 2126, line 1-4, first column). Applicants were attempting to express their invention in the awkward terminology of Hansbrough *et al.* The nonporous "planar surface" of Hansbrough *et al.* is at the upper boundary of their biosynthetic substratum and supports the upper portion of the composite. Applicants conceived the Hansbrough *et al.* invention as thereby defining an upper "plane" (better termed a stratum, as planes obviously lack width) consisting essentially of cultured human keratinocytes isolated from the fibroblasts by the nonporous surface. The previous response was simply trying to point out that in contrast to Hansbrough *et al.* who teach a composite wherein the keratinocytes and fibroblasts are segregated on different sides of a nonporous layer, Applicants' claimed invention locates keratinocytes and fibroblasts on the same upper side of the 'plane' or, more particularly, the surface defined by the upper boundary of the biosynthetic substratum. As indicated in the rest of the previous response and throughout the amended claims therein, as well as in the specification as filed (see for instance the description of Fig. 1A on p. 6), within that upper surface region the keratinocytes and fibroblasts may be further stratified so that the keratinocytes are in a layer over the fibroblasts.

In light of the above, Applicants respectfully request that the above rejection be reconsidered and withdrawn.

3. **Claims 4-6**

Claims 4-6 stand rejected as allegedly indefinite with respect to the recital of "said second dermal fibroblast layer." Applicants believe their amendments to claims 4-6 moot this issue and respectfully request that this rejection be reconsidered and withdrawn.

4. **Claims 7 and 14**

Claims 7 and 14 stand rejected as allegedly indefinite with respect to the recital of "a substratum of a biosynthetic substratum." Applicants have canceled claims 7 and 14 thus rendering this rejection moot.

5. **Claim 8**

Claim 8 stands rejected as indefinite in the recital of "compromising." The claim has been amended to recite therefor "comprising." Applicants respectfully request that this rejection be reconsidered and withdrawn.

6. **Claim 16**

Claim 16 stands rejected as allegedly indefinite in its recitation of "an upper side of a biosynthetic substratum." Claim 16 has been canceled thus mooting this rejection.

C. **Response to the Rejections under 35 U.S.C. §102(b)**

1. **Claims 1, 7, 8, and 14**

Claims 1, 7, 8, and 14 stand rejected as allegedly anticipated by Della Valle *et al.* for reasons of record advanced on pp. 4-5 of the Office Action of Paper 4. Claims 7 and 14 have been canceled to expedite prosecution of the application.

Pursuant to MPEP §2131, to anticipate a claim, a reference must disclose all the elements of the claim. The Della Valle *et al.* reference does not anticipate all the elements of claims 1 and 8. Applicants have amended claim 1 to recite:

> 1. A method for cultivating a graftable skin material for application to a human patient, said method comprising:
>
> growing a layer human dermal fibroblasts upon an upper side of a biosynthetic substratum of an esterified hyaluronic

> acid; and, after said dermal fibroblast layer begins to proliferate,
>
> growing a layer of human keratinocytes harvested from said patient over said dermal fibroblasts upon said upper side of said substratum to form said graftable skin material, said material thereby comprising viable human dermal fibroblasts and viable human keratinocytes on said upper side.

Della Valle *et al.* do not teach the use of *human* fibroblast cells as recited in claim 1. Della Valle *et al.* teach the use of *murine* 3T3 feeder cells. Della Valle *et al.* do not teach the use of *viable* fibroblasts. Della Valle *et al.* teach the use of *irradiated* fibroblasts (see col. 4, line 20) and *lethally irradiated 3T3* fibroblasts (see col. 4, line 40). Della Valle *et al.*'s artificial skin is not described as even retaining the 3T3 fibroblasts. Retention of the xenofibroblasts would be undesirable in application of Della Valle *et al.*'s material to humans. Presumably, Della Valle *et al.*'s 3T3 cells are removed when "all traces" of the culture medium are removed by a wash with a physiological solution (col. 5, lines 50-55). Claim 1 as amended recites "said material thereby comprising viable human dermal fibroblasts and viable human keratinocytes on said upper side." Della Valle *et al.*'s artificial skin therefore does **not** have any fibroblasts. Clearly, Della Valle *et al.* do not disclose the use of human or viable fibroblasts in their disclosure.

Claim 8 as amended is also drawn in part to fibroblasts which are human and viable. Therefore, Della Valle *et al.* do not anticipate the subject matter of claim 8.

Applicants therefore respectfully request that the above rejections of claims 1 and 8 be reconsidered and withdrawn.

D. Response to the Rejections under 35 U.S.C. §103(b)

Rejections in View of Della Valle *et al.* and Hansbrough *et al.*

Claims 2, 3, 9, and 10 stand rejected as allegedly obvious in view of Della Valle *et al.* (1997) and Hansbrough *et al.* (1989) for reasons of record advanced on p. 6 of the previous Office Action of Paper 4. Applicants respectfully traverse this rejection:

1. The proposed combination changes the principle of operation of the primary reference.

Pursuant to MPEP §2143.01, "the proposed modification cannot change the principle of operation of a reference." The Action cited Hansbrough *et al.* "only for providing

evidence that it was well-established in the art to use autologous fibroblasts and keratinocytes in preparing graftable skin material." Here, the suggested combination of the Della Valle *et al.* and Hansbrough *et al.* references would require a substantial reconstruction and redesign of the elements shown in the primary Della Valle reference as well as a change in the basic principle under which the primary Della Valle *et al.* reference construction was designed to operate. Della Valle *et al.* teach how to grow an epithelial sheet. Della Valle *et al.* teach use of irradiated 3T3 fibroblasts to help the keratinocytes to grow on the biosynthetic substrate to form a skin graft material. However, the skin graft product produced thereby essentially consists of the keratinocytes and biosynthetic substratum, not fibroblasts (see col. 5, lines 30-40). The 3T3 fibrocytes are xenogenic and not fairly characterized as an integral component of the final skin graft. Incorporating human *viable* fibroblasts into the artificial skin product of Della Valle *et al.* is a substantial reconstruction and redesign of the Della Valle *et al.* product.

In view of the above, Applicants respectfully request that the above proposed combination be reconsidered and withdrawn.

2. The proposed combination fails to teach all the elements of the claims.

In addition, pursuant to MPEP § 2143, in order to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The proposed combination of the Della Valle *et al.* and Hansbrough *et al.* references does not teach all the limitations of the claims:

The Action cited Hansbrough *et al.* "only for providing evidence that it was well-established in the art to use autologous fibroblasts and keratinocytes in preparing graftable skin material." Applicants submit that substitution of the autologous human fibroblasts and keratinocytes of Hansbrough *et al.* for the 3T3 cells and keratinocytes of Della Valle *et al.* would not provide all the elements of the present claims. Della Valle *et al.* teach use of fibroblasts to help the keratinocytes grow on the biosynthetic substrate to form the skin graft material. However, the skin graft material of Della Valle *et al.* essentially consists of the

keratinocytes and biosynthetic substratum, **not** the fibroblasts (see col. 5, lines 30-40). The Della Valle *et al.* fibrocytes are not fairly characterized as an integral component of the final skin graft. The Della Valle *et al.* graft material has no upper or basal side (see col. 5, line 54). Substitution of irradiated human fibroblasts would not result in the inventive compositions or methods.

In view of the above, Applicants respectfully request that the above proposed combination be reconsidered and withdrawn.

3. The prima facie case is also rebutted by teaching away from the prior art.

A *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention. *In re Geisler*, 116 F.3d 1465, 1471, 43 USPQ2d 1362, 1366 (Fed. Cir. 1997). Here, the prior art teaches away from the proposed combination. The Zacchi *et al.* reference previously cited by the Examiner helps to explain why no one had attempted the subject matter of the rejected claims 2, 3, 9, and 10 by growing or providing "a layer of human dermal fibroblasts upon an upper side of a biosynthetic substratum of an esterified hyaluronic acid" as recited in their corresponding base claims, claims 1 and 8. In the paragraph bridging pp. 187 and 187, Zacchi *et al.* discuss the need for a three dimensional supporting matrix for cultured fibroblasts:

> "A major problem in culturing dermal structure in "ex vivo" is the fact that cultured fibroblasts, in common with most cells, grow in two dimensions. For this reason, a three dimensional dermal architecture can not be achieved. To overcome this difficulty, three dimensional structures have been used as supports for fibroblast growth, such as collagen-glycosaminglycan matrices, allogenic dermis, and synthetic polymers."

Indeed, in every cited reference in which fibroblast containing artificial skin is provided, the graft fibroblasts are grown using a three-dimensional matrix. Accordingly, one of ordinary skill in the art had no reasonable expectation that growing fibroblasts on the "upper side" of a substratum would work.

In view of the above, Applicants respectfully request that the above proposed combination be reconsidered and withdrawn.

E. Rejections in view of Della Valle *et al.* (1997) in view of Cooper *et al.* (1993), Hansbrough *et al.* (1989), and Meyers *et al.* (1977)

Claims 11-13 stand rejected as allegedly obvious over Della Valle *et al.* (1997) in view of Cooper *et al.* (1993), Hansbrough *et al.* (1989), and Myers *et al.* (1977).

The Cooper *et al.* reference is essentially redundant of the Hansbrough *et al.* reference and the Della Valle *et al.* reference. Cooper *et al.* and Hansbrough *et al.* both disclose a composite graftable material having a collagen-GAG dermal substrate containing human fibroblasts covered by a non-porous laminate on which human keratinocytes are seeded. Cooper *et al.* and Della Valle *et al.* both disclose a laser skin keratinocyte product produced using *feeder* 3T3 cells. In comparing the take rates for both the composite material and the laser skin keratinocyte product, the Cooper *et al.* reference treats them as distinct entities and provides no suggestion to combine their features. Aside perhaps for the use of autologous keratinocytes, the Myers *et al.* reference is basically redundant of the Della Valle reference as it principally also teaches the use a laser skin seeded keratinocyte material prepared using *feeder* 3T3 cells. In Myers *et al.*, the material is applied in some instances to a dermal wound bed, but the graftable material itself does not include that dermal component. Thus, the above proposed combination is essentially redundant of the first proposed combination and subject to traversal for the same reasons.

Independent claim 11 has been amended to recite:

> 11. A graftable skin material for application to a patient, said materal comprising a composite of:
>
> a biosynthetic substratum of an esterified hyaluronic acid;
>
> a first layer of viable human dermal fibroblasts upon a basal side of said biosynthetic substratum;
>
> a second layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and
>
> a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from said patient.

Thus, as amended, independent claim 11 recites a composition which, as discussed above, is not taught or suggested by any of the above cited references. There is simply no teaching or suggestion of "a second layer of viable human dermal fibroblasts upon an upper

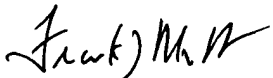
side of said biosynthetic substratum." As such, Applicants request that the above rejections of claims 11-13 be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



Frank J. Mycroft
Reg. No. 46,946

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (415) 576-0200
Fax: (415) 576-0300
FJM
WC 9041487 v1

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

A. The last full paragraph on page 1 has been amended to recite:

[Alloderm™ and Integra™] ALLODERM™ dermal matrix and INTEGRA™ Artificial Skin are two currently popular examples of human skin substitute commercially available in the market. AlloDerm™ is donated human tissue that is processed to remove all epidermal and dermal cells while preserving the remaining biological dermal matrix. [Integra™] INTEGRA™ Artificial Skin, a brand of artificial skin, is sold by Integra LifeScience Corporation of Plainsboro, New Jersey, USA, and has been approved by FDA for use in the USA since 1996. [Artificial skin] INTEGRA™ Artificial Skin is a bilayer biosynthetic sheet comprising porous collagen-glycoaminoglycan integrated with a thin silicone membrane as an outer layer. The use of [artificial skin such as Integra] such a porous collagen-glycoaminoglycan artificial skin as a biocompatible acellular dermal replacement in deep and full-thickness burn wounds is well known.

B. The paragraph bridging pages 1 and 2 has been amended to recite:

It has been observed that within about 14 to 21 days following the grafting of [Integra] a bilayer biosynthetic sheet comprising porous collagen-glycoaminoglycan integrated with a thin silicone membrane as an outer layer, there is full vascularization of the neodermis formed in the [Integra] artificial skin. Thereafter an ultra thin split thickness skin graft must be harvested from a donor site in order to cover the neodermis immediately after the silicone membrane is removed. Substantial research effort has been undertaken in the past to determine the possibility of reliably grafting CEA on the neodermis, since an effective combination of CEA and [Integra] such a dermal replacement as a biosynthetic collagen-glycoaminoglycan dermal matrix should eliminate the second operative stage, the associated pain and scaring, as well as a need for a second donor site, which may not be available in extensively burned patients. If the grafted CEA does not 'take' on the neodermis [of Integra] after the silicone membrane is peeled off, it can be replaced by another CEA. Whereas, in the conventional application of [Integra] such a biosynthetic collagen-glycoaminoglycan dermal matrix dermal replacement, another split thickness autograft must be harvested from a second or even a third donor site. There have been very limited initial anecdotal reports on

experience with such a combination technique, such as Sheridan *et al.* 1999 and Pandya *et al.* 1998. At the 10th Congress of International Society For Burn Injuries, November 1998 in Israel, the difficulties with the conventionally cultured graft anchoring onto [the neodermis of Integra] such a biosynthetic collagen-glycoaminoglycan neodermis were addressed. The exact reasons for such difficulties remain unknown.

C. The first full paragraph on page 2 has been amended to recite:

[Laserskin™ material is a thin] LASERSKIN™ artificial skin is another artificial skin material. This material is made of a thin (e.g., about 20 μm-thick membrane) and pliable biosynthetic membrane comprising a 100% benzyl esterified hyaluronic acid derivative suitable for use as a substratum in the growth of skin cells. The membrane is drilled by a laser to have a series of holes or microholes typically of about 40 μm in diameter to allow the ingrowth and proliferation of keratinocytes. In addition, a series of larger holes (about 0.5 mm in diameter) are also typically provided to allow drainage of wound fluids. LASERSKIN™ artificial skin is available from Fida Advanced Biopolymers Ltd. (Abano Terme, Italy). The recommendation of the LASERSKIN™ artificial skin manufacturer is to seed human keratinocytes on [Laserskin] the LASERSKIN™ artificial skin material preseeded with irradiated 3T3 cells as feeder layer. When [following the manufacturer's recommendation] seeding human keratinocytes on LASERSKIN™ artificial skin preseeded with irradiated 3T3 cells as feeder layer, it was found that, after the initiation of the formation of keratinocyte colonies, the xenogenic 3T3 cells growing on the [Laserskin] material were less likely to be washed away than those growing on a culture dish as in the conventional Green's method during each flushing procedure with phosphate-buffered saline (Rheinwald and Green, Cell, 6:331-344 (1975)). It is believed that the remaining 3T3 cells or debris might have sensitized the host to xenogenic antigen resulting in undesired late graft rejection. What is needed is a cultivation and engraftment procedure with a biocompatible, durable human skin substitute.

D. The first paragraph of the Summary on page 4 has been amended to recite:

[According to the invention, autologous cultured keratinocytes grown on a biocompatible substratum are engrafted on the neodermis of artificial skin covering a wound. Autologous keratinocytes may be cultivated on a commercially available membrane such as Laserskin™ (available from Fidia Advanced Biopolymers Ltd., Abano Terme (PD), Italy)

following pre-seeding with autologous or allogenic dermal fibroblasts. The resultant composite material may then be applied on the neodermis of artificial skin which had been previously engrafted on the patient. The composite material, and specifically Composite Biocompatible Skin Graft (CBSG) material comprises autologous keratinocytes and allogenic or autologous dermal fibroblasts grown on the substratum. A method for fabricating the composite material includes the application of dermal fibroblasts onto the substratum as a feeder layer and then inoculating autologous keratinocytes on the resultant structure. A method for engraftment comprises first applying an artificial skin with a protective silicone membrane on a wound area, thereby allowing vascularization; following vascularization, removing the silicone membrane and engrafting the cultured composite material onto the vascularized artificial skin.]

--According to the invention, autologous cultured keratinocytes grown on a biocompatible substratum are engrafted on the neodermis of artificial skin covering a wound. In one of its aspects the invention provides a method for cultivation of graftable skin by growing a layer of dermal fibroblasts upon at least an upper side of a biosynthetic substratum of a derivative of benzyl esterified hyaluronic acid; and, after the dermal fibroblast layer grows to become at least sub-confluent, growing a layer of keratinocytes from cells harvested from the intended recipient/target donor patient over these dermal fibroblasts to form a composite skin graft material.

In another of its aspects, the invention provides a method for the cultivation of a graftable skin by growing a layer of keratinocytes from cells harvested from the intended recipient/target donor patient upon an upper side of a biosynthetic substratum of a derivative of benzyl esterified hyaluronic acid to form a composite skin graft material.

In another of its aspects, the invention provides a graftable skin material comprising a composite of a biosynthetic substratum of a derivative of benzyl esterified hyaluronic acid; a layer of dermal fibroblasts upon at least an upper side of the biosynthetic substratum; and a layer of keratinocytes grown from cells harvested from the intended recipient over the dermal fibroblasts upon the upper side of the substratum.

In another of its aspects, the invention provides a graftable skin material comprising a composite of: a biosynthetic substratum of a derivative of benzyl esterified hyaluronic acid; a first layer of dermal fibroblasts upon a first basal side of the biosynthetic substratum; a second layer of dermal fibroblasts upon a second upper side of the biosynthetic

substratum; and a layer of keratinocytes grown from cells harvested from the intended recipient over the dermal fibroblasts grown upon the upper side of the substratum.

In another aspect, the invention provides a method for grafting a graftable skin material by applying an artificial skin substrate upon a wound bed of a recipient patient; the artificial skin substrate comprising a layer of collagen-glycoaminoglycan on a basal side to be juxtaposed to said wound bed and a covering membrane of silicone on an opposing upper side; allowing a sufficient time to form a vascularized wound bed under the collagen-glycoaminoglycan; thereupon removing the silicone membrane; and thereupon applying a basal side of a sheet of cultivated skin material over said collagen- glycoaminoglycan, said cultivated skin material comprising at least a layer of keratinocytes upon an upper side of a substratum, said keratinocytes being harvested from a target donor patient. In one embodiment, the cultivated skin material further comprises a layer of dermal fibroblasts upon at least an upper side of a biosynthetic substratum in which the layer of keratinocytes is over the dermal fibroblasts. In another embodiment, another layer of dermal fibroblasts is located upon the basal side of the biosynthetic substratum.

In each of the above aspects, the dermal fibroblasts may be allogenic or autologous to the keratinocytes.

Autologous keratinocytes may be cultivated on a biosynthetic membrane following pre-seeding with autologous or allogenic dermal fibroblasts. One such biosynthetic membrane comprises a 100% benzyl esterified hyaluronic acid derivative having a series of laser drilled holes or microholes typically of about 40 um in diameter to allow the ingrowth and proliferation of keratinocytes and a series of larger holes (about 0.5 mm in diameter) to allow drainage of wound fluids (LASERSKIN™ artificial skin commercially available from Fidia Advanced Biopolymers Ltd., Abano Terme (PD), Italy). The resultant composite material may then be applied on the neodermis of artificial skin which had been previously engrafted on the patient. The composite material, and specifically Composite Biocompatible Skin Graft (CBSG) material comprises autologous keratinocytes and allogenic or autologous dermal fibroblasts grown on the substratum. A method for fabricating the composite material includes the application of dermal fibroblasts onto the substratum as a feeder layer and then inoculating autologous keratinocytes on the resultant structure. A method for engraftment comprises first applying an artificial skin with a protective silicone membrane on a wound area, thereby allowing vascularization; following vascularization, removing the silicone

membrane and engrafting the cultured composite material onto the vascularized artificial skin.--

E. The paragraph bridging pages 4 and 5 has been amended to recite:

Human fibroblasts used in the cultivation technique according to the invention were found to achieve a role similar to 3T3 cells in the initiation of keratinocyte colonies on [Laserskin] LASERSKIN™ artificial skin. Specifically, it was found that the seeding efficacy of human keratinocytes was increased to up to 95%. CBSG containing autologous keratinocytes and autologous dermal fibroblast or allogenic dermal fibroblasts or a combination of autologous and allogenic dermal fibroblasts according to the invention, has been successfully applied to burn patients whose wounds were previously grafted with allografts.

F. The second full paragraph on page 5 has been amended to recite:

CBSG material according to the invention offers notable advantages. First, the basal proteins (including the early basement membrane proteins such as collagen IV and fibronectin) of the cultured graft are protected from dispase treatment because the keratinocytes are directly cultivated on [a] the pliable [Laserskin] laser drilled membrane. This is believed to enhance anchorage of the cultured keratinocytes on the neodermis [of Integra]. Second, in addition to acting as a feeder layer, the dermal fibroblasts in the inventive CBSG material evidently produce a number of proteins such as native collagen fibers and fibronectin which is believed to facilitate the attachment of a cultured graft. Third, the cultured keratinocytes of the inventive CBSG can be grafted five to seven days sooner than can traditionally-cultured keratinocytes. This is because cultured keratinocytes of the inventive CBSG are capable of being transferred and grafted at the sub-confluent or less differentiated stage rather than at a later confluent stage. Fourth, since there is minimal need for a donor site there is less likelihood of widespread scarring related to donor site harvesting. Fifth, the cultured keratinocytes of CBSG can be handled much more easily than the conventional CEA during its application on the neodermis of the artificial skin. Fewer cultured cells are lost or damaged during the transfer and application of CBSG. This should improve the success rate of the cultured graft. Sixth, the inventive engraftment technique can result in higher demand and broader scope of clinical applications for artificial skin.

G. The heading and paragraph between lines 11 and 19 on page 7 have been amended to recite:

[Laserskin] Biosynthetic biocompatible substratum

[Laserskin] In the following experiments, LASERSKIN™ artificial skin was used. LASERSKIN™ artificial skin is a biosynthetic biocompatible substratum for keratinocyte cultivation according to the invention. [It] LASERSKIN™ artificial skin is a form of thin and pliable biosynthetic membrane comprising a 100% benzyl esterified hyaluronic acid derivative. This material is drilled with holes to facilitate the culturing of the keratinocytes thereupon. [Laserskin] LASERSKIN™ artificial skin is commercially available from Fidia Advanced Biopolymers Ltd., Abano Terme (PD), Italy. [Laserskin] LASERSKIN™ artificial skin was found to be useful to the inventors' experiments, although its preparation and application are not done according to the manufacturer's conventional instructions. There is nothing to preclude the use of compatible bioequivalents or human skin substitutes would also work in a similar fashion with the inventive CBSG.

H. The heading at the bottom of page 7 has been amended to recite:

Comparison of seeding efficacy of human keratinocytes on [Laserskin] LASERSKIN™ artificial skin

I. All the paragraphs on page 8 have been amended to recite:

Human dermal fibroblasts and 3T3 cells were grown separately on [Laserskin] LASERSKIN™ artificial skin with DMEM supplemented with 10% FBS. At sub-confluency, the 3T3 cells were treated with mitomycin-C (4mg/ml) for 2 hours at 37 °C, whereas the human fibroblasts were not treated. Human keratinocytes were seeded ($3x10^4$ cells/cm^2) on top of the [Laserskin] LASERSKIN™ artificial skin either pre-seeded with 3T3 cells or human fibroblasts. The keratinocyte suspension was instilled and concentrated on the Laserskin surface (0.5ml/cm^2). Thirty minutes after seeding, DMEM supplemented with 10% FBS, insulin (4mg/ml), cholera toxin (6ng/ml), and EGF (10ng/ml) (per Rheinwald *et al.* 75) were added to the culture.

In a control run, human keratinocytes were also seeded on top of plain [Laserskin] LASERSKIN™ artificial skin (Figure 1C). Efficacy was exhibited as hereinafter noted. After incubation at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours, the non-seeded keratinocytes on the [Laserskin] LASERSKIN™ artificial skin were gently rinsed off

with PBS. The cells were then counted with a hemocytometer. In order to quantify the number of fibroblasts or 3T3 cells detached together with the non-seeded keratinocytes during the washing of the [Laserskin] LASERSKIN™ artificial skin, the [Laserskin] LASERSKIN™ artificial skin without keratinocytes but seeded with equivalent number of 3T3 cells or fibroblasts were included as control.

Comparison of seeding efficacy of rat keratinocytes on [Laserskin] LASERSKIN™ artificial skin

The seeding of rat keratinocytes on [Laserskin] LASERSKIN™ artificial skin was performed according to a technique essentially the same as the seeding of human keratinocytes. Rat fibroblasts were used to replace the human fibroblasts.

Preparation of CBSG graft for use on full-thickness wounds

Three different types of composite skin graft (CBSG) materials were compared and tested in animals, specifically laboratory rats. Type A CBSG consisted of allogenic rat fibroblasts seeded onto the basal side of a [Laserskin] LASERSKIN™ artificial skin substratum. The fibroblasts were stimulated to produce collagen and other proteins by feeding the fibroblasts with DMEM supplemented with 10% FBS and 50μm ascorbic acid. After ten days, the [Laserskin] LASERSKIN™ artificial skin substratum was turned over and the upper side was seeded with fibroblasts. The fibroblasts on both sides were then fed with DMEM supplemented with 10% FBS. When the fibroblasts on the upper side became sub-confluent, rat keratinocytes were seeded ($3x10^4$ cells/cm^2) on top of the upper surface of CBSG material. The cells were fed with DMEM supplemented with 10% FBS, insulin (4mg/ml), cholera toxin (6ng/ml), EGF(10ng/ml). The keratinocytes became sub-confluent and were ready for grafting after only four to six days.

J. The paragraph bridging pages 9 and 10 and the first three paragraphs of page 10 have been amended to recite:

Results

Twenty-four hours after the seeding on the CBSG substratum, the non-viable and unseeded keratinocytes were carefully washed away and concentrated for counting. There were no detectable 3T3 cells or fibroblasts detached from the [Laserskin] LASERSKIN™

artificial skin during the washing procedure. The seeding efficacy of keratinocytes was calculated as:

$$\frac{[\text{Total keratinocytes count (before seeding)} - \text{non-seeded keratinocytes}]}{\text{Total keratinocytes count (before seeding)}} \times 100\%$$

It is evident that the selected type of CBSG substratum provided a suitable culture template for the in vitro proliferation of keratinocytes. On a plain [Laserskin substratum] LASERSKIN™ artificial skin, human keratinocytes showed a mean seeding efficacy of 75%. These human keratinocytes had a 95% seeding efficacy on [Laserskin] LASERSKIN™ artificial skin populated with human fibroblasts and a 98% on 3T3 cell-seeded [Laserskin] LASERSKIN™ artificial skin (Table 1). Rat keratinocytes had a seeding efficacy of 36% on plain [Laserskin] LASERSKIN™ artificial skin. The respective seeding efficacies of rat keratinocytes on 3T3/[Laserskin]LASERSKIN™ artificial skin and on allogenic fibroblasts/[Laserskin]LASERSKIN™ artificial skin were 91% and 88%. The seeding efficacies of human/rat keratinocytes growing on 3T3 cell/[Laserskin]LASERSKIN™ artificial skin or on allogenic fibroblasts/[Laserskin]LASERSKIN™ artificial skin were significantly ($p < 0.001$) better than those seeded on plain [Laserskin] LASERSKIN™ artificial skin, as noted in the Fisher Exact Testing using Stat Xact (version 2.05) statistical package.

It is believed that human/rat fibroblasts could achieve a role similar to that of the 3T3 cells in enhancing the seeding efficacies of keratinocytes growing on [Laserskin] LASERSKIN™ artificial skin with respective p values of 0.445 and 0.646 using the same statistical package.

The optical transparency of the [Laserskin] LASERSKIN™ artificial skin allowed regular inspection of the grafted wound bed as it healed. Skin biopsies were taken from the center of the grafted area of the subject. It was observed that the polypropylene ring prevented the migration of epithelium from the wound edge as no epithelial cell was found in the control rat wound sutured with polypropylene ring alone up to day 21. In sixteen out of the twenty (80%) animal wounds covered with Type A CBSG, the keratinocytes formed a multi-layered epithelium that had a basal layer in contact with underlying connective tissue. The undersurface of epidermis did not show rete. Fibrovascular ingrowth of connective tissue into the [Laserskin] LASERSKIN™ artificial skin was observed. Eight (40%) of the

Type B CBSG sites and seven (35%) of the Type C CBSG sites showed re-epithelization. Histologically there was no observable epithelium in 3 (15%), 10 (50%), and 12 (55%) of the grafted CBSG sites of Types A, B and C respectively (Table 2).

K. The paragraphs of the Discussion section from line 12, page 11 through line 10, page 12 have been amended to recite:

Problems encountered in cultivation of keratinocytes on [Laserskin type material] LASERSKIN™ artificial skin can be attributed to limited experience on this new product. Hyaluronic acid is a mucopolysaccharide with alternating β 1-3 glucuronidic and β 1-4 glycosaminidic bonds. [Laserskin] LASERSKIN™ artificial skin mainly consists of 100% benzyl esterified hyaluronic acid. A weak electrostatic association exists between the keratinocytes and the [Laserskin] LASERSKIN™ artificial skin. Since the volume and surface area of human keratinocytes differ from those of rat keratinocytes, there is a variation of charge density of two cell populations. Consequently they have different seeding efficacies on plain [Laserskin] LASERSKIN™ artificial skin. The species-specific difference was abolished by cultivating keratinocytes on 3T3 cell/[Laserskin]LASERSKIN™ artificial skin because 3T3 cells were the dominant factors influencing keratinocyte attachment on [Laserskin] LASERSKIN™ artificial skin.

The seeding efficacies of human and rat keratinocytes (3×10^4 cells/cm^2) were 75% and 36% respectively on plain [Laserskin] LASERSKIN™ artificial skin. The manufacturer of LASERSKIN™ artificial skin recommends seeding the human keratinocytes on [Laserskin] the skin membrane pre-seeded with irradiated 3T3 cells. Good seeding efficiencies of 98% (human keratinocytes) and 91% (rat keratinocytes) were demonstrated on [Laserskin] LASERSKIN™ for artificial skin with Mitomycin-treated 3T3 cells. In Green's method, keratinocytes are seeded on a 3T3 cell feeder layer which allows a rather low seeding density of $6\text{-}10 \times 10^4$ cells/cm^2 for the primary culture. A smaller seeding density of $2\text{-}5 \times 10^4$ cells/cm^2 is feasible for the secondary and tertiary cultures. The utility of cultured epidermal autograft grown on culture dish using Green's feeder layer technique is limited by the persistent 3T3 fibroblasts which sensitize the host to xenogenic antigen resulting in late graft rejection. The 3T3 cells were found less likely to be washed away from the [Laserskin] LASERSKIN™ artificial skin material. However, allogenic fibroblasts can achieve a similar role as 3T3 cells in initiation of keratinocyte colonization on [Laserskin] LASERSKIN™ artificial skin.

It is evident that the CBSG material consisting of cultured keratinocytes and dermal fibroblasts is a good human skin substitute for freshly excised full-thickness wounds which were previously grafted with allografts.

LASERSKIN™ Artificial Skin Preparation	Human Keratinocytes	Rat keratinocytes
Plain [Laserskin]	75%	36%
[Laserskin] with 3T3 cells	98%	91%
[Laserskin] with human fibroblasts	95%	-
[Laserskin] with rat fibroblasts	-	88%

Table 1 Mean Seeding Efficacies of Human and Rat Keratinocytes on [Laserskin] LASERSKIN™ artificial skin. 15 determinations were made. Seeding efficacies of human/rat keratinocytes were significantly improved by 3T3 cells/fibroblasts ($p < 0.001$).

L. **The paragraphs from line 4 on page 13 through line 10 on page 14 have been amended to recite:**

Attempts were made to graft freshly harvested epidermis onto [the neodermis of Integra] INTEGRA™ neodermis in animal experiments. The dermal-epidermal separation was performed with dispase. The epidermal graft was not easily 'taken' by the neodermis whereas thin split thickness autografts readily incorporated on the neodermis as in many clinical applications. It is believed that the presence of fibroblasts in the dermis of the thin autograft may play an essential role in the keratinocyte attachment and its proliferation on the neodermis. It was observed that, during wound healing, cell-cell interactions between epidermal keratinocytes and dermal fibroblasts contributed to the organization of epidermis. Prostaglandin E2 (PGF2) is considered to be involved in the proliferation and differentiation of keratinocytes. The production of PEG2 was enhanced in the co-culture of keratinocytes and fibroblasts, whereas the PEG2 production was negligible in monolayer cultures of keratinocytes or fibroblasts. The fibroblasts also produced two soluble heparin-binding growth factors such as keratinocyte growth factor and hepatocyte growth factor/scatter factor that promote DNA synthesis and proliferation of keratinocytes.

The combination of the inventive CBSG material including keratinocytes and dermal fibroblasts and [Integra] INTEGRA™ artificial skin material was applied to a 28-year

old man with extensive hypertrophic burn scars and neck contracture. Multiple wounds from scar excision with a total area of approximately 250 cm^2 were covered with [Integra] INTEGRA™ artificial skin material. At the same time a skin biopsy was taken for keratinocyte culture. Two weeks later, the top silicone layer of the artificial skin was removed. The neodermis of the artificial skin was then covered with the CBSG. At Days 6 and 14 after the application of CBSG, biopsies were taken from center of one of the wounds. The histology at Day 6 showed early epithelization with a few squamous epithelial cells settling on top of the scaffolds of [the Integra] INTEGRA™ artificial skin material and [Laserskin] the LASERSKIN™ artificial skin material. Most of the epithelium were single-layer flattened squamous cells. The biopsy taken on Day 14 showed a viable 2- to 4-cell-thick squamous epithelium with minimal inflammation. The wounds were completely epithelized within four weeks of the application of the CBSG. Biopsies taken from other wounds showed similar findings.

In the claims:

Claims 7, 14, and 16 have been canceled without prejudice.

Claims 1, 4, 8, 11, and 15 have been amended to recite as follows:

1. (Twice Amended) A method for cultivating a graftable skin material for application to a human patient, said method comprising:

growing a layer of human dermal fibroblasts upon an upper side of a biosynthetic substratum of an esterified hyaluronic acid; and, after said dermal fibroblast layer begins to proliferate,

growing a layer of human keratinocytes harvested from said patient over said dermal fibroblasts upon said upper side of said substratum to form [a composite skin graft material, said keratinocytes being harvested from a target donor patient] said graftable skin material, said material thereby comprising viable human dermal fibroblasts and viable human keratinocytes on said upper side.

4. (Twice Amended) A method for cultivating graftable skin material for application to a human patient, said method comprising:

growing a first layer of human dermal fibroblasts upon a basal side of a biosynthetic substratum of an esterified hyaluronic acid;

growing a second [layer of dermal fibroblasts] human dermal fibroblast layer upon an upper side of said biosynthetic substratum; and

after said second dermal fibroblast layer begins to proliferate, growing a layer of keratinocytes over said [dermal fibroblasts upon said upper side of said substratum] second layer to form a composite skin material, said keratinocytes having been harvested from [a target donor] said patient.

8. (Twice Amended) A graftable skin material for application to a patient, said material [compromising] comprising a composite of:

a biosynthetic substratum of an esterified hyaluronic acid;

a layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from [a target donor] said patient.

11. (Twice Amended) A graftable skin material for application to a patient, said material comprising a composite of:

a biosynthetic substratum of an esterified hyaluronic acid;

a first layer of viable human dermal fibroblasts upon a basal side of said biosynthetic substratum;

a second layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from [a target donor] said patient.

15. (Twice Amended) A method for grafting a graftable skin material onto a human patient, comprising the steps of:

applying an artificial skin substrate upon a wound bed of [a recipient] said patient; said artificial skin substrate comprising a layer of collagen-glycoaminoglycan on a basal side to be juxtaposed to said wound bed and a covering membrane of silicone on an opposing upper side;

allowing a vascularized wound bed to form under said collagen-glycoaminoglycan; thereupon

removing said silicone membrane; and

applying a basal side of a sheet of cultivated skin material over said collagen-glycoaminoglycan, said cultivated skin material comprising a layer of keratinocytes overlying a layer of viable human dermal fibroblasts upon an upper side of a biosynthetic substratum, said keratinocytes being harvested from [a target donor] said patient.

The following new claims 18-30 have been added:

18. (New) A method according to claim 15, wherein said biosynthetic substratum is a substratum of an esterified hyaluronic acid.

19. (New) The method according to claim 18 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon an upper side of said biosynthetic substratum and wherein said layer of keratinocytes is over said dermal fibroblasts.

20. (New) The method according to claim 19 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon said basal side of said biosynthetic substratum.

21. (New) The method of claim 1, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.

22. (New) The method of claim 4, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.

23. (New) The material of claim 8, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

24. (New) The material of claim 11, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

25. (New) The method of claim 18, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

26. (New) The method of claim 21, wherein the membrane has microholes of about 40 μm diameter.

27. (New) The method of claim 22, wherein the membrane has microholes of about 40 μm diameter.

28. (New) The material of claim 23, wherein the membrane has microholes of about 40 μm diameter.

29. (New) The material of claim 24, wherein the membrane has microholes of about 40 μm diameter.

30. (New) The method of claim 25, wherein the membrane has microholes of about 40 μm diameter.

In the Abstract:

The Abstract has been amended to recite:

Autologous cultured keratinocytes are grown on a biosynthetic and biocompatible substratum following pre-seeding with autologous or allogenic dermal fibroblasts. The resultant composite material may then be applied on the neodermis of artificial skin which had been previously engrafted on the patient. The composite material, and specifically Composite Biocompatible Skin Graft (CBSG) material comprises autologous keratinocytes and allogenic or autologous dermal fibroblasts grown on [Laserskin] an artificial skin. A method for cultivating the CBSG includes the application of dermal fibroblasts onto the substratum as a feeder layer and then the inoculation of autologous keratinocytes on the resultant structure. A method for engraftment comprises first applying an artificial skin with a protective silicone membrane on a wound area, thereby allowing vascularization; and following vascularization, removing the silicone membrane and engrafting the CBSG material onto the vascularized artificial skin.

In the drawings:

The legends for the Photographs of Figures A1 through A3 in Appendix A have been amended to recite:

Fig. A1 is a depiction of in vitro proliferation of keratinocytes on plain [Laserskin.] LASERSKIN™ artificial skin showing holes for drainage of exudate (x100).

Fig. A2 is a depiction of a Type A modified Composite Biocompatible Skin Graft (CBSG) according to the invention wherein allogenic fibroblasts were seeded on both sides of the [Laserskin] LASERSKIN™ artificial skin (x400).

Fig. A3 is a depiction of a Type B modified Composite Biocompatible Skin Graft (CBSG) according to the invention wherein fibroblast was seeded only on the side opposite the basal side of the [Laserskin] LASERSKIN™ artificial skin (x400).

APPENDIX I

PENDING CLAIMS AFTER ENTRY OF THIS AMENDMENT

1. A method for cultivating a graftable skin material for application to a human patient, said method comprising:

growing a layer of human dermal fibroblasts upon an upper side of a biosynthetic substratum of an esterified hyaluronic acid; and, after said dermal fibroblast layer begins to proliferate,

growing a layer of human keratinocytes harvested from said patient over said dermal fibroblasts upon said upper side of said substratum to form said graftable skin material, said material thereby comprising viable human dermal fibroblasts and viable human keratinocytes on said upper side.

2. The method according to claim 1 wherein said dermal fibroblasts are allogenic to the keratinocytes.

3. The method according to claim 1 wherein said dermal fibroblasts are autologous to the keratinocytes.

4. A method for cultivating graftable skin material for application to a human patient, said method comprising:

growing a first layer of human dermal fibroblasts upon a basal side of a biosynthetic substratum of an esterified hyaluronic acid;

growing a second human dermal fibroblast layer upon an upper side of said biosynthetic substratum; and

after said second dermal fibroblast layer begins to proliferate, growing a layer of keratinocytes over said second layer to form a composite skin material, said keratinocytes having been harvested from said patient.

5. The method according to claim 4 wherein said dermal fibroblasts are allogenic to the keratinocytes.

6. The method according to claim 4 wherein said dermal fibroblasts are autologous to the keratinocytes.

8. A graftable skin material for application to a patient, said material comprising a composite of:

a biosynthetic substratum of an esterified hyaluronic acid;

a layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from said patient.

9. The material according to claim 8 wherein said dermal fibroblasts are allogenic to the keratinocytes.

10. The material according to claim 8 wherein said dermal fibroblasts are autologous to the keratinocytes.

11. A graftable skin material for application to a patient, said material comprising a composite of:

a biosynthetic substratum of an esterified hyaluronic acid;

a first layer of viable human dermal fibroblasts upon a basal side of said biosynthetic substratum;

a second layer of viable human dermal fibroblasts upon an upper side of said biosynthetic substratum; and

a layer of viable human keratinocytes over said dermal fibroblasts upon said upper side of said substratum, said keratinocytes having been harvested from said patient.

12. The material according to claim 11 wherein said dermal fibroblasts are allogenic to the keratinocytes.

13. The material according to claim 11 wherein said dermal fibroblasts are autologous to the keratinocytes.

15. A method for grafting a graftable skin material onto a human patient, comprising the steps of:

applying an artificial skin substrate upon a wound bed of said patient; said artificial skin substrate comprising a layer of collagen-glycoaminoglycan on a basal side to be

juxtaposed to said wound bed and a covering membrane of silicone on an opposing upper side;

allowing a vascularized wound bed to form under said collagen-glycoaminoglycan; thereupon

removing said silicone membrane; and

applying a basal side of a sheet of cultivated skin material over said collagen-glycoaminoglycan, said cultivated skin material comprising a layer of keratinocytes overlying a layer of viable human dermal fibroblasts upon an upper side of a biosynthetic substratum, said keratinocytes being harvested from said patient.

17. The method according to claim 16 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon said basal side of said biosynthetic substratum.

18. (New) A method according to claim 15, wherein said biosynthetic substratum is a substratum of an esterified hyaluronic acid.

19. (New) The method according to claim 18 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon an upper side of said biosynthetic substratum and wherein said layer of keratinocytes is over said dermal fibroblasts.

20. (New) The method according to claim 19 wherein said cultivated skin material further comprises a layer of dermal fibroblasts upon said basal side of said biosynthetic substratum.

21. (New) The method of claim 1, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.

22. (New) The method of claim 4, wherein the esterified hyaluronic acid is benzyl esterified hyaluronic acid.

23. (New) The material of claim 8, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

24. (New) The material of claim 11, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

25. (New) The method of claim 18, wherein the substratum is a membrane comprising benzyl esterified hyaluronic acid.

26. (New) The method of claim 21, wherein the membrane has microholes of about 40 μm diameter.

27. (New) The method of claim 22, wherein the membrane has microholes of about 40 μm diameter.

28. (New) The material of claim 23, wherein the membrane has microholes of about 40 μm diameter.

29. (New) The material of claim 24, wherein the membrane has microholes of about 40 μm diameter.

30. (New) The method of claim 25, wherein the membrane has microholes of about 40 μm diameter.